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Microsatellites reveal origin and genetic diversity of Eurasian invasions by one of the world's most notorious marine invader, *Mnemiopsis leidyi* (Ctenophora)

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Abstract

Marine invasions are taking place at an increasing rate. When occurring in blooms, zooplanktivorous comb jellies of the genus *Mnemiopsis* are able to cause pelagic regime shifts in coastal areas and may cause the collapse of commercially important fish populations. Using microsatellites, developed for the first time in the phylum Ctenophora, we show that *Mnemiopsis leidyi* has colonized Eurasia from two source regions. Our preliminary data set included four sites within the putative source region (US East Coast and Gulf of Mexico) and 10 invaded locations in Eurasian waters. Bayesian clustering and phylogeographic approaches revealed the origin of earlier invasions of the Black and Caspian Sea in the 1980s/1990s within or close to the Gulf of Mexico, while the 2006 invasion of the North and Baltic Seas can be directly traced to New England (pairwise F_{ST} = 0). We found no evidence for mixing among both gene pools in the invaded areas. While the genetic diversity (allelic richness) remained similar in the Baltic Sea compared to the source region New England, it was reduced in the North Sea, supporting the view of an initial invasion of Northern Europe to a Baltic Sea port. In Black and Caspian Sea samples, we found a gradual decline in allelic richness compared to the Gulf of Mexico region, supporting a stepping-stone model of colonization with two sequential genetic founder events. Our data also suggest that current practices of ballast water treatment are insufficient to prevent repeated invasions of gelatinous zooplankton.

Keywords: Bayesian clustering, founder event, gelatinous plankton, marine invasion, *Mnemiopsis leidyi*

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Introduction

Biological invasions continue to homogenize biota in all environments. This applies particularly to marine coastal areas which are among the ecosystems harbouring the highest proportion of non-indigenous species (Carlton & Geller 1993; Cohen & Carlton 1998; Grosholz 2002). Although most invasive species have little apparent impact on native biodiversity and ecosystem processes, some may completely alter food web structure of the invaded areas (Ricciardi & Kipp 2008). Comb jellies of the genus *Mnemiopsis* (Phylum: Ctenophora) provide a key example. In their native range along the US Atlantic coastline they are known as voracious predators that can control the secondary production of planktonic crustaceans, especially in the absence of their natural predators (Kremer 1994). In the invaded areas of Black, Azov and Caspian Sea, abundant *Mnemiopsis*

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leidyi A. Agassiz 1865 have contributed to major ecological regime shifts from a pelagic system dominated by planktivorous fish to one dominated by gelatinous plankton, including a total collapse of the pelagic fisheries in the 1990s (Shiganova & Bulgakova 2000; Kideys *et al.* 2005; Oguz *et al.* 2008). The recent discoveries of *M. leidyi* in the North Sea (Faasse & Bayha 2006; Boersma *et al.* 2007), off the Norwegian Coast (Oliveira 2007), in the Baltic Sea (Javidpour *et al.* 2006) and in the NW Mediterranean (Fuentes *et al.* 2009) have thus caused major concern among marine ecologists and ecosystem managers, in particular, because ctenophore abundances were in a range that may deplete zooplankton populations (Javidpour *et al.* 2009).

Identification of the invasion pathways and source populations constitute critical first steps in the understanding of success, impact and eventually management of an invasion event (Sax *et al.* 2007). Evidently, a successful management of invasion vectors is only possible if the invasion routes and source populations and localities are identified (Grosholz 2002; David & Gollasch 2008). Moreover, an identification of the source of invasive genotypes is mandatory for inferences on evolutionary adaptation during an invasion processes (Lee 2002; Sax *et al.* 2007). For example, in a matching genotype scenario (Suarez & Tsutsui 2007), source individuals coming from the Gulf of Mexico would be pre-adapted to a warm temperate location as the Black Sea, but not to the cold-temperate Baltic, while the opposite would be true for source genotypes coming from New England. Finally, as part of a risk assessment, the possibility of hybridization resulting from multiple introductions needs to be evaluated, as the merger of previously separated source gene pools in their novel habitat can increase the ecological performance of invasive populations (Ellstrand & Schierenbeck 2000; Lee 2002; Sax *et al.* 2007).

The questions of source locale and putative multiple invasions can be most effectively addressed using high-resolution genetic markers such as DNA microsatellites or single nucleotide polymorphisms (SNPs). Such markers are often the only means to understand the transport of invasive species when the vector is ship ballast water, as hypothesized in the case of *M. leidyi*, which has many possible origins and target locales (David & Gollasch 2008; Kölzsch 2009). The application of high-resolution genetic markers along with novel statistical tools, for example methods that detect population structure in non-ideal populations (Pritchard *et al.* 2000), have been very instructive in tracking marine invasion pathways (Andreakis *et al.* 2009; Brawley *et al.* 2009) and in detecting repeated introductions (Roman 2006; Darling *et al.* 2008) and subsequent population hybridizations (Voisin *et al.* 2005; Darling *et al.* 2008).

Here, we analyse the population genetic structure of the recent invasion of *M. leidyi*, one of the most successful invasive species worldwide, to North and Baltic Sea where this species was first recorded in 2006 (Faasse & Bayha 2006; Javidpour *et al.* 2006; Boersma *et al.* 2007) We develop DNA microsatellite markers for these species, which to our knowledge are the first such markers for the phylum Ctenophora. Based on polymorphism at these loci, we then identify the likely source and invasion route of older (Black Sea, Caspian) and more recent (Baltic, North Sea) invasions of *M. leidyi* from NW Atlantic coastal waters. Second, we determine whether or not the genetic diversity of the invading populations is reduced compared to the source populations (Sax & Brown 2000; Allendorf & Lundquist 2003) Third, we assess whether or not the invasion from different source regions has already resulted in hybridizations among previously separated populations, a factor which has been implicated as an important process determining the success of an invasion (Ellstrand & Schierenbeck 2000; Nolte *et al.* 2005).

Materials and methods

Sample collection

The sample set contained 467 *M. leidyi* individuals from a total of four putative source and 10 invaded locations (Table 1). The samples were collected during 2008 and 2009 by plankton tows or by surface dipping. Samples were preserved by drying onto filter paper (glass fibre or cellulose) at ambient temperature.

Genetic methods

Crude DNA was extracted using the Invitek kit for animal tissue (Invitek, Germany), using a 0.5 cm × 0.5 cm piece of filter. In order to confirm the taxonomic affiliation of *M. leidyi* throughout its source region and within the invaded areas, we employed sequences of the internally transcribed spacer (ITS1 and 2) of the rDNA as a taxonomic marker. We sequenced the 18S-ITS1 region using primers suggested by Gorokhova *et al.* (2009). Another subset of samples was sequenced for the ITS1 and ITS2 region using primer pairs described by White *et al.* (1990). DNA sequences in 23 specimen from nine sampling locations were obtained using Sanger sequencing with BigDye (v.3.1) chemistry on an ABI 3130 genetic analyzer. Trace files (forward and reverse) were inspected by eye and aligned manually in BioEdit.

For high resolution analysis of donor populations and invasion pathways, we utilized the polymorphism of microsatellite loci. We developed seven microsatellite primer pairs based on an enriched genomic library and

Table 1 Sampled locations, geographic position and sample size for *Mnemiopsis leidyi* in the USA (source region) and Eurasia (invaded areas)

Sampling site	Invasion/sampling year	Abbreviation	rDNA sequencing	*N*	Co-ordinates	Collector (Institution)
Black Sea Bulgaria	~1985/2009	BSB	Yes	15	43°11′N, 27°57′ E	Kremena Stefanova (Bulgarian Institute of Oceanology)
Black Sea Turkey	~1985/2009	BST		23	42° 01′ N, 35°08′ E	Levent Bat (Sinop University)
Black Sea Ukraine	~1985/2009	BSU		48	44°37′N 33°31′E	Alexandra Gubanova (Institute of Biology of the Southern Seas)
Caspian Sea	1999/2008	CAS	Yes	22	36°48′N, 53° 07′E	Jamileh Javidpour (IFM-GEOMAR)
Fehmarn Belt	2006/2009	FEM		48	54°30′N, 11°20′E	Sören Bolte (IFM- GEOMAR)
Kristineberg	2006/2009	KRS		37	58°24′N, 11°24′E	Lene Fries Möller (University of Gothenburg)
Helgoland	2006/2009	HEL	Yes	46	54°11′N, 07°53′E	Philipp Schubert (IFM- GEOMAR)
Kiel Fjord	2006/2009	KIF	Yes	21	54°25′N, 10°12′E	Sören Bolte (IFM- GEOMAR)
Bornholm Basin	2006/2009	BBA	Yes	48	55°11′N, 15°32′E	Bastian Huver (DTU Aqua)
Maasholm	2006/2008	MAH	Yes	42	54̊41′N, 10̊00′E	Maxi Sparwel (Uni Münster/IFM- GEOMAR)
USA Woods Hole	Native/2009	USA-WH	Yes	45	41° 31′N, 70°40′W	Woods Hole specimen service
USA Panacea	Native/2009	USA-PC	Yes	33	30°00′N, 84°20′W	Jack Rudloe (Gulf Specimen Marine Lab)
USA Port Aransas	Native/2009	USA-PA		19	27°50′N, 97°03′W	Anthony G. Moss (Auburn University)
USA Galveston Bay	Native/2009	USA-GB	Yes	20	29°17′N, 94°52′W	Anthony G. Moss (Auburn University)

on existing EST-data (see Data S1, Supporting information, for a full description of microsatellite development and characterization). These primers amplify loci that possess between 5 and 48 alleles (total 204 alleles) across all 14 sampling sites (Data S1, Supporting information). For genotyping, fluorescently labelled PCR products were electrophoretically separated on an ABI3130 sequencer using the POP7 polymer and sized using the internal standard Rox350 (Applied Biosystems). The software GeneMapper (Applied Biosystems) was used to define size classes of alleles and to semi-automatically genotype all specimen in the complete data set. The microsatellite markers (including primer sites) are deposited in GenBank under accession numbers HM147251–HM147256.

Data analysis

In order to detect structure among all of the detected genotypes without any a priori assumptions regarding the sampled 'populations', we employed a Bayesian clustering algorithm implemented within the software Structure (Pritchard *et al.* 2000). The most likely number of clusters (*K*) was inferred using the method proposed by Evanno *et al.* (2005) which considers the shape of the log-likelihood function with increasing *K*. We used the genetic admixture model and the option of correlated allele frequencies between populations because of the possible presence of linkage disequilibrium. We performed 10 000 reiterations of the burn in and 100 000 MCMC (Markov chain Monte Carlo) repetitions for each of six replicate tests ranging from a number of clusters of $K = 1$ to $K = 7$.

Calculation of pairwise and global F_{ST} values, permutation tests and calculation of heterozygosities and allelic richness were performed using Genetix 4 (Belkhir *et al.* 1998). In order to calculate the number of alleles we first excluded all sample locations with less than 20 genotypes [USA (Galveston Bay) and Bulgaria (Black Sea)]. The sample size was then standardized to the same number of individuals ($N = 20$) by rarefaction (10 resampling runs). We tested for deviations from Hardy-Weinberg equilibrium using exact tests implemented into the web-version of the software Genepop (Rousset 2008).

In order to reveal the topology of phylogeographic relatedness among sampled locations, we computed a neighbour-joining (NJ) tree based on Cavalli-Sforza's and Edwards chord distance. Choosing this distance measure has been shown to have the highest likelihood to reveal the true topology when using microsatellite frequency data (Takezaki & Nei 1996). The tree topology was examined using 1000 bootstrap runs of allele frequencies prior to calculation of the distance matrix. These procedures were performed in the Phylip package v. 3.69, using the subprograms Seqboot, dnadist,

NEIGHBOUR, CONSENSE and DRAWTREE (Felsenstein 1989, 2009). Only bootstrap support >60% was further considered at any branching point.

Results

Sequencing of the ITS region of rDNA

Sequences of rDNA were obtained from 21 specimen collected at nine locations (Table 1, GenBank accession numbers HM147257–HM147277), and compared to two published sequences of the 18S-ITS1-5.8S–ITS2-28S region (Podar *et al.* 2001; Faasse & Bayha 2006). Except for a single polymorphism in the ITS1 region and two polymorphisms in the ITS2 region, the sequences obtained in this study were similar to one another (Table 2). They also closely resembled the only sequence data deposited for the genus *Mnemiopsis* (*M. leidyi*) in Genbank from the Woods Hole area (Podar *et al.* 2001) and from the Netherlands (Faasse & Bayha 2006). None of the observed polymorphisms revealed any systematic distribution among the genetic clusters identified using DNA microsatellites (see below). All coding region (parts of 18S and 5.8S rDNA) were completely identical among one another. The full alignment can be found in Data S2 (Supporting information).

Table 2 Polymorphic sites in the ITS1–5.8S–ITS2 region in 21 *M. leidyi* individuals from nine locations (this study) and compared to existing Genbank accessions (topmost two sequences). A dash (–) indicates no sequence data at this residue. The first base pair of ITS1 is at position 1. The full sequence alignment can be found in Data S2 (Supporting information)

Location, sample no.	Accession number	ITS1 285	ITS2 489	ITS2 508
USA—Woods Hole†	AF293700	C	A	T
The Netherlands‡	EF175463	C	–	–
USA—Woods Hole_01	HM147257	C	T	C
USA—Woods Hole_16	HM147272	C	–	–
USA—Panacea_22	HM147273	T	–	–
USA—Panacea_03	HM147274	C	–	–
USA—Galveston Bay02	HM147275	–	T	–
Bornhom Bassin_01	HM147258	C	T	C
Bornholm Bassin_11	HM147259	C	T	C
Bornholm Bassin_12	HM147260	C	T	C
Bornholm Bassin_13	HM147261	C	T	C
Helgoland_052	HM147262	C	T	C
Helgoland_122	HM147263	–	T	T
Helgoland _82	HM147264	C	T	C
Helgoland _32	HM147265	C	T	C
Maasholm_02	HM147266	C	T	C
Maasholm _01	HM147267	T	T	T
Maasholm _05	HM147268	C	A	T
Maasholm _04	HM147269	C	T	C
Kiel Fjord_07	HM147270	C	-–	–
Kiel Fjord_09	HM147271	T	–	–
CaspianSea_15	HM147276	T	–	–
CaspianSea_06	HM147277	C	–	–

†Podar *et al.* (2001).
‡Faase & Bayha (2006).

Microsatellite analysis and population structure

Along with the sampling locations, the allele frequencies of the two least polymorphic microsatellite loci (MnleC1583 and MneleL13) are presented in Fig 1. The qualitative notion of one major genetic subdivision among the 14 sampling locations into a 'southern' and a 'northern' group was subsequently quantified and statistically tested in population genetic analyses. The STRUCTURE analysis revealed the most significant increase in log-likelihood from K = 1 to K = 2 clusters (data not shown) while thereafter, the log-likelihood remained unchanged and the variance increased. According to Evanno *et al.* (2005), we concluded that K = 2 is the most likely number of genetic clusters (Fig. 2). The first of these two subgroups comprises one of the putative source regions, New England (Woods Hole) and all recently invaded North Sea, Skagerrak and Baltic Sea sites (hereafter 'northern' cluster NewEngland/NorthSea/Baltic). A second subgroup consisted of another putative source region, the Gulf of Mexico (three sites) and of the older invaded locations in Black Sea and Caspian Sea (hereafter 'southern' cluster Gulf/Black/CaspianSea). No further resolution of both subsets of the data was possible using the Bayesian clustering algorithm implemented in STRUCTURE (data not shown). In support of this pattern based on six microsatellite loci, a seventh locus (L211) that amplified perfectly in all New England/North Sea/Baltic samples, often failed in the PCR of genotypes from the southern clade. Notwithstanding these results, among the 23 alleles that revealed frequencies ≥10% in either the southern or northern clade, 17 were shared among the two major population subdivisions, suggesting that both gene pools are not completely separated (see Fig. 1 e.g. loci MnleC1583 and MneleL13).

This major genetic subdivision is supported by the tree topology based on allele frequencies using Cavalli Sforza's and Edwards chord distance (Fig. 3). In 1000/1000 bootstrap runs, the same two clusters as above were resolved. Within the northern cluster, additional genetic subdivision could be detected. The topology of the NJ-tree resolved a small but significant differentiation between North Sea/Skagerrak and the Baltic Sea sites (Fig. 3). This divergence was supported by applying Wright's *F*-statistics, with pairwise differentiation data of

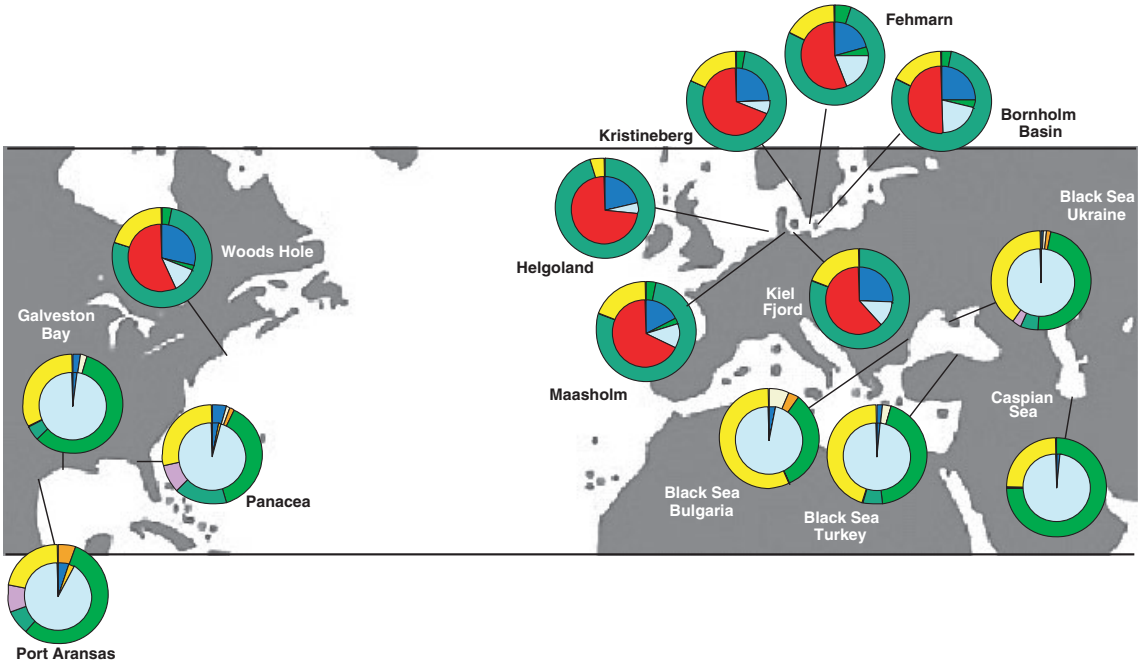

Fig. 1 Sampling locations of *M. leidyi* in their native distribution range along the North American Coast and in Eurasia (invaded locations). Pie-diagrams depict allele frequencies of two microsatellite loci that display five alleles (inner circle, MnleC1583) and seven alleles (MnleL13, outer circle), respectively. Note the overlap in the common alleles which suggests that both gene pools are not completely seperated.

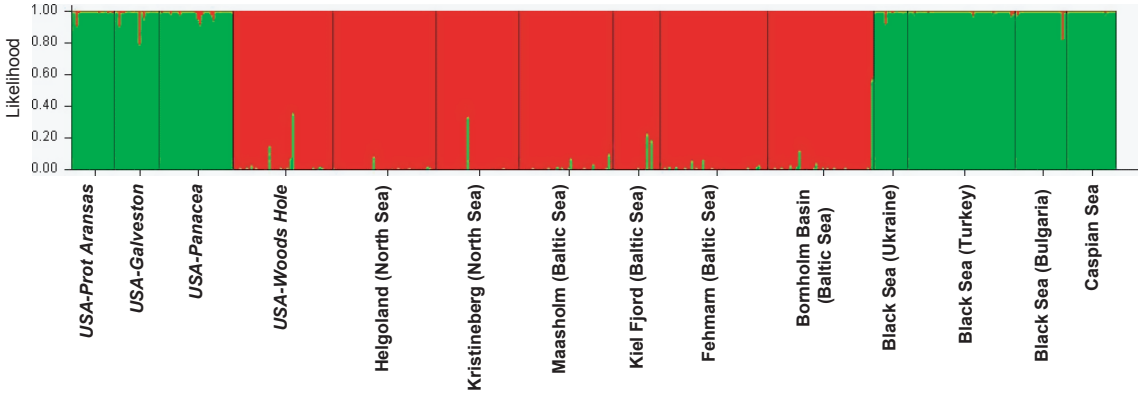

Fig. 2 Bar diagram of Bayesian clustering of *M. leidyi* genotypes from 14 locations using the software STRUCTURE (Pritchard *et al.* 2000). Different colours represent both major genetic clusters. A red bar represents the likelihood of the genotype to be tightly clustered with the 'northern' Baltic/NorthSea/New England group; the green represents the 'southern' Gulf/Black Sea/Caspian group. Sorting along the x-axis was performed according to the sampling locations with the width of each site proportional to the number of animals collected.

up to F_{ST} = 0.031 ($P < 0.01$) among all possible pairs North Sea–Baltic Sea (Table 3).

Genetic substructuring was also found within the southern cluster (Gulf/Black/Caspian Sea), with three additional branches shown in the tree topology that had significant bootstrap support (Fig. 3). The matrix of F_{ST} values indicated that all Gulf of Mexico sites were significantly differentiated from the *M. leidyi* samples from the Black/Caspian Sea (all pairwise F_{ST} >0.033, Table 3). These differences were small relative to the major difference between northern and southern clade (F_{ST} ~0.3), but nevertheless statistically highly significant (all $P_{permutation} < 0.001$). The chord distance tree provided weak support for the Caspian Sea being more closely related to the Gulf of Mexico as the putative source region (Fig. 3). Also worth mentioning is the relatively high genetic differentiation among the three Black Sea sites, both in the tree topology (Fig. 3) and in the pairwise F_{ST} values (F_{ST} = 0.008–0.057, Table 3).

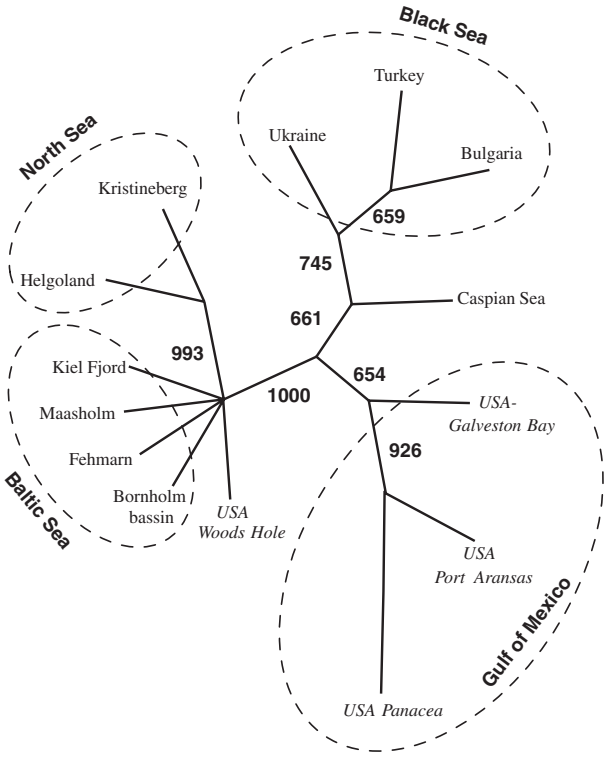

Fig. 3 Neighbour-joining tree based on microsatellite allele frequencies in 14 population samples of *M. leidyi*. The distance matrix is based on Cavalli-Sforza's chord distance and was bootstrapped 1000 times.

Genetic diversity

Almost all populations showed a deficit of heterozygote individuals relative to Hardy-Weinberg proportions (Table 4). For detecting genetic founder events, we focus here on the average number of alleles/locus per population (allelic richness). The more recent invasion to North and Baltic Sea did not result in a loss of genetic diversity in the Baltic Sea samples (Maasholm, Kiel Fjord, Fehmarn, Bornholm Bassin) in terms of allelic richness (standardized to a common number of 20 sampled individuals, Fig. 4a and b). Within the northern cluster, the same applied to heterozygosity patterns (observed and expected, Table 4). Interestingly, we found that both North Sea sites (Helgoland, Kristineberg) have a significantly lower observed heterozygosity and allele number than the four Baltic Sea sites (*t*-test, df = 4, $P = 0.004$ and 0.001, respectively, Fig. 4a).

In the southern cluster the situation is different (Fig. 4b). Here, putative source populations in the Gulf of Mexico (Port Aransas and Panacea) showed the highest allelic richness, while the genetic diversity is progressively decreasing in the order Black Sea (primary invasion) → Caspian Sea (secondary invasion). In the heterozygosity data (observed and expected), no such trend could be observed (graph not shown, Table 4).

Discussion

As one process contributing to the 'jellyfication' of the world's oceans, invasions have recently received growing attention (Richardson *et al*. 2009). One prime example are ongoing invasions by the comb jelly *M. leidyi* that have contributed to a corresponding regime shift in coastal seas dominated by secondary consumption of gelatinous zooplankton rather than fish. We demonstrate here that *M. leidyi* came to Eurasia by two clearly distinct events, one during the 1980s/1990s with a source region within or close to the Gulf of Mexico and a more recent invasion in 2006 originating from New England. In general, the amount of population genetic

Table 3 Population structure in *M. leidyi*. Pairwise genetic differentiation (above diagonal) and associated *P*values (1000 permutations, lower diagonal) among native (italics) and 10 non-indigenous populations, calculated according to Weir & Cockerham (1984), implemented in GENETIX. Negative F_{ST} values were set to zero. For population abbreviations see Table 1

	USA-PA	*USA-GB*	*USA-PC*	*USA-WH*	HEL	KRS	MAH	KIF	FEM	BBA	BSB	BSU	BST	CAS
USA-PA		0.000	0.000	0.242	0.321	0.282	0.253	0.253	0.226	0.230	0.051	0.067	0.044	0.068
USA-GB	0.60		0.009	0.268	0.349	0.309	0.281	0.284	0.253	0.257	0.053	0.068	0.032	0.075
USA-PC	0.61	0.09		0.230	0.305	0.270	0.242	0.241	0.216	0.218	0.046	0.063	0.042	0.080
USA-WH	<0.001	<0.001	<0.001		0.030	0.015	0.006	0.000	0.002	0.003	0.257	0.273	0.295	0.305
HEL	<0.001	<0.001	<0.001	0.001		0.003	0.027	0.021	0.031	0.029	0.348	0.349	0.378	0.383
KRS	<0.001	<0.001	<0.001	0.16	0.16		0.016	0.006	0.015	0.016	0.303	0.313	0.339	0.345
MAH	<0.001	<0.001	<0.001	0.49	<0.001	<0.001		0.000	0.001	0.006	0.271	0.284	0.309	0.315
KIF	<0.001	<0.001	<0.001	0.08	0.002	0.15	0.9		0.000	0.000	0.275	0.291	0.317	0.330
FEM	<0.001	<0.001	<0.001	0.14	<0.001	<0.001	0.11	0.48		0.000	0.246	0.259	0.282	0.291
BBA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.09	0.51	0.87		0.247	0.260	0.283	0.294
BSB	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		0.043	0.008	0.070
BSU	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		0.056	0.094
BST	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.19	<0.001		0.087
CAS	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	

Table 4 Genetic diversity (observed and expected heterozygosity) in native and invasive populations of *M. leidyi* quantified at six microsatellite loci. All populations except Black Sea—Bulgaria showed a significant deficit in heterozygote individuals (exact test in GENEPOP, $P < 0.001$)

Population	H_{exp} †	H_{obs}
USA—Port Aransas	0.6778	0.4950
USA—Galveston Bay	0.6160	0.3659
USA—Panacea	0.6863	0.5689
USA—Woods Hole	0.7537	0.6638
Helgoland (North Sea)	0.6429	0.5619
Kristineberg (North Sea)	0.6991	0.5868
Maasholm (Baltic Sea)	0.7364	0.6572
Kiel Fjord (Baltic Sea)	0.7392	0.6786
Fehmarn (Baltic Sea)	0.7665	0.6699
Bornholm Basin (Baltic Sea)	0.7623	0.6861
Black Sea Bulgaria	0.6167	0.4566
Black Sea Ukraine	0.6212	0.6201
Black Sea Turkey	0.5470	0.4402
Caspian Sea	0.5352	0.4394

substructuring encountered in a holo-planktonic species such as a ctenophore that can be transported by ocean currents was surprising (Kinlan & Gaines 2003) and facilitated the identification of the invasion source. We also refute the idea that the North Sea/Baltic Sea are invaded from *M. leidyi* individuals with Ponto-Caspian origin, as is the case with several other prominent examples [Round Goby, Corkum *et al.* (2004); zebra mussel, Nalepa & Schloesser (1993)].

According to some studies, *M. leidyi* is supposed to occur only north of Cape Hatteras (Kremer 1994) while individuals collected in the Gulf of Mexico are within the distribution range of a sister species, *Mnemiopsis mccradyi* (Mayer 1900). If true, the earlier invasion to Black and Caspian Sea in the 1980s and 1990s would be attributable to *M. mccradyi* Mayer 1900 (Zaika & Sergeyeva 1990). However, the majority of researchers conclude that *M. leidyi* has invaded the Caspian and Black Sea (e.g. Shiganova & Bulgakova 2000; Kideys *et al.* 2005; Oguz *et al.* 2008), which is supported by our preliminary analysis of rDNA sequences. None of the few polymorphisms in the ITS1 and 2 regions were reciprocally fixed among both major microsatellite supported clades. In addition, we find considerable overlap in the occurrence of the most common alleles across both clades, suggesting that some genetic exchange must have occurred in the recent past (Fig. 1). However, a definite answer on the status of *Mnemiopsis* species along the West-Atlantic coastline would require more DNA sequence data from additional genes, which was beyond the scope of this study. The importance of genetic methods in identifying gelatinous plankton and the potential invasion status of comb jellies has recently been highlighted by Gorokhova

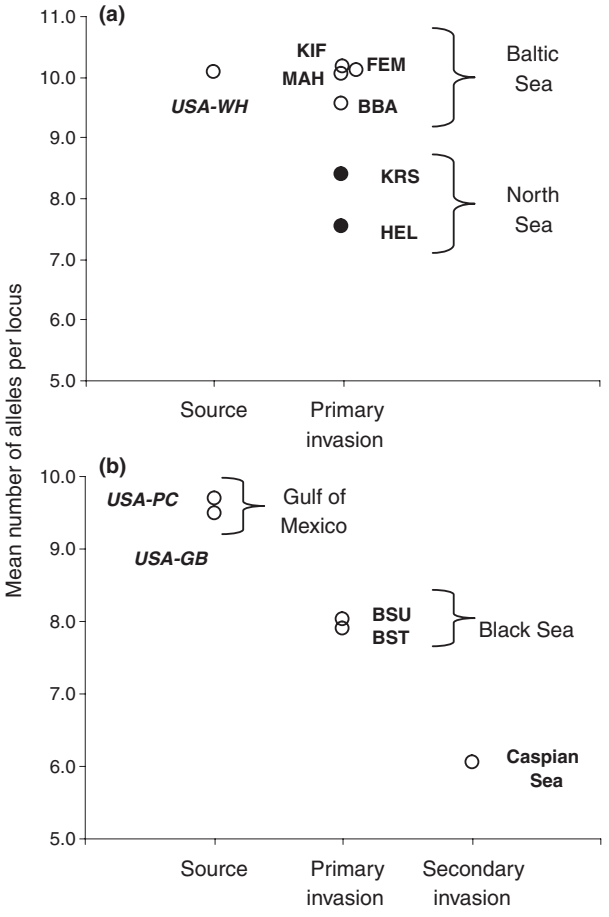

Fig. 4 Comparison of mean allelic richness (six loci) in *M. leidyi* among putative source and invaded locations. Sampling locations were ordered by inferred invasion sequence. The sample size per location was standardized to $N = 20$ via rarefaction. Panel (a) compares the New England location to North Sea (filled circles) and Baltic Sea (open circles) sites. Note that in panel (a) the North Sea invasion may be an unresolved secondary invasion via the Baltic Sea. Panel (b) depicts the progressive decline in the order Gulf region sites → Black Sea locations → Caspian Sea. For population abbreviations see Table 1.

et al. (2009) who identified another invasive comb jelly, *Mertensia ovum*, in the northern Baltic Sea, which was previously misidentified as *M. leidyi*. This study identified the Bornholm basin as the northernmost location where *M. leidyi* was unambiguously identified using ITS sequencing and microsatellites.

The 'southern' genetic cluster Gulf/Black/Caspian Sea revealed a complex pattern of genetic substructure. All three putative source locations were not significantly differentiated from one another (Table 3) and thus represent a quite extended, panmictic gene pool that was the source for individuals transported to the Black Sea/Caspian Sea in the 1980s. The time that has elapsed since then led to the divergence in microsatellite

frequencies within a single enclosed sea (Black Sea), with pairwise differentiations up to $F_{ST} = 0.057$.

In the course of the more recent invasion, an interesting finding was that North Sea sites (Helgoland and Kristineberg) are weakly but significantly differentiated from the four sites in the Baltic (pairwise differentiation of North Sea–Baltic Sea of $F_{ST} \sim 0.03$; all $P < 0.001$, Table 3). These findings are in line with the chord-distance cladogram, where New England (Woods Hole) is closer to all Baltic Sea sites than to both North Sea from the Baltic sites (Fig. 3). As the most parsimonious scenario, an almost direct transport of individuals from the New England area brought *M. leidyi* to the Baltic Sea (pairwise $F_{ST} = 0$). The North Sea was either colonized secondarily from the Baltic Sea or received an independent propagule pool of smaller size directly from the US East coast. We can exclude that the recent 2006-invasion of the Baltic Sea was a 'spill-over' from the adjacent North Sea. This finding is counter-intuitive because most direct shipping routes between New England and Europe end in Hamburg (Germany) and Rotterdam (The Netherlands) (Kölzsch 2009).

In the same vein, the 1980s colonization of the Black Sea and later Caspian Sea (1990s) probably happened in a stepping stone fashion, supported by the progressive loss of allelic richness from the Black Sea to the Caspian Sea. However, the topology of the cladogram (based on chord distance) provides weak evidence that there may have been influx from the Gulf region directly to the Caspian Sea (Fig. 3). It all boils down to the need for a denser spatial and temporal sampling over longer time intervals, a goal that we will pursue as we continue to examine the more recent invasions of North Sea and Baltic Sea.

The 'paradox of invasions' (Sax & Brown 2000; Allendorf & Lundquist 2003) posits that invasive populations by definition are ecologically successful, although the concomitant founder event is expected to reduce genetic variation. However, several recent examples suggest that invasive populations may actually harbour a higher genetic diversity than in their native range. This is most likely to occur when genotypes from several sources are mixed or when the inoculum size is large (Kolbe *et al.* 2004; Voisin *et al.* 2005; Roman 2006). One major goal of this study was therefore to investigate whether past and recent invasions resulted in the loss of genetic diversity in *M. leidyi*. To this end, heterozygosity and allelic richness can be taken into account. Inferences based on heterozygosity values can be problematic when populations show signs of deviation from Hardy-Weinberg-equilibrium, which may partly come from null-alleles or inbreeding. Even selfing is possible in *M. leidyi*, as this species is a simultaneous hermaphrodite (Martindale 1987). In contrast, estimates using allelic richness per population are certainly more robust estimates of neutral genetic diversity. It is also known that the number of alleles is a more sensitive indicator of population bottlenecks than heterozygosity (Piry *et al.* 1999). Therefore, we focus on allelic richness, which was standardized by resampling to $N = 20$ individuals per location.

Accordingly, in *M. leidyi*, the recent invasion to the Baltic Sea was clearly not accompanied by losses of genetic diversity at marker loci, in line with other recent studies on marine invasions (Voisin *et al.* 2005; Roman & Darling 2007; Andreakis *et al.* 2009). We found that along the invasion of the northern genetic cluster, the genetic diversity (number of alleles and observed heterozygosity) in the Baltic was similar to New England (Woods Hole). However, in the North Sea, marker diversity was markedly lower compared to New England, which suggests that the genotypes found in the North Sea are a spill-over from the Baltic Sea, in line with the data on genetic structure (Fig. 3). The interpretation of the diversity data of the earlier invasion to Black Sea/Caspian Sea is easier. Here, we do find a progressive loss in allelic richness in the invaded area compared to the Gulf of Mexico sites, suggesting two consecutive founder events. In summary, within the same species, the genetic diversity was apparently reduced during an older invasion wave (Black Sea/Caspian Sea), while in another more recent one it differs between invaded areas (Baltic Sea vs. North Sea).

We found no evidence for mixing of both major gene pools in the invaded areas. Because such hybridization may release additive genetic variance (Lee 2002), may increase the vigour of invasive genotypes (Ellstrand & Schierenbeck 2000; Suarez & Tsutsui 2007), and even trigger speciation events (Nolte *et al.* 2005) further secondary transport of *M. leidyi* within Eurasia that may bring both genetic clusters into contact should be avoided at all costs. In order to evaluate the potential for within-species hybridization and its ecophysiological consequences, we will examine whether population mixing exists between northern and southern *M. leidyi* populations along the NW Atlantic coastline.

To conclude, we identified a second completely independent invasion of a notorious invader, the comb jelly *M. leidyi*, that may cause large and unprecedented regime shifts of pelagic food webs in northern European Seas. As such, our results provide additional motivation to efficiently manage ballast water on a faster timeline as envisaged by the International Maritime Organization (David & Gollasch 2008).

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Supporting information

Additional supporting information may be found in the online version of this article.

Data S1 (for Reusch *et al.* 2010): Microsatellites reveal origin and genetic diversity of Eurasian invasions by one of the world's most notorious marine invader, *Mnemiopsis leidyi* (Ctenophora).

Data S2 Alignment of DNA sequences of the 18S-ITS1-5.8S-ITS2 region in *Mnemiopsis* spp. from nine locations, three native (Woods Hole—New England; Gulf of Mexico—Panacea, and Galveston Bay) and six invaded ones.

Table S1 Seven microsatellite primers in the comb jelly *M. leidyi*

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